

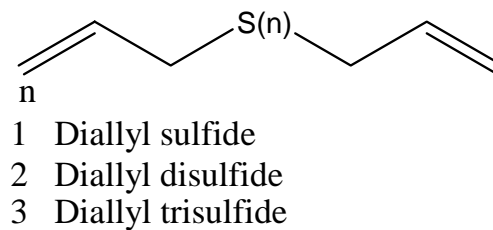
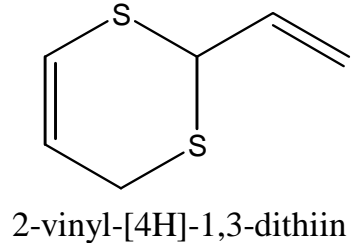
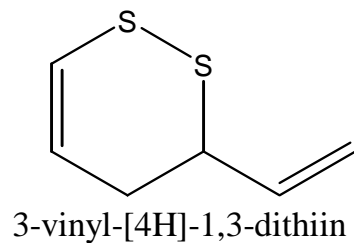
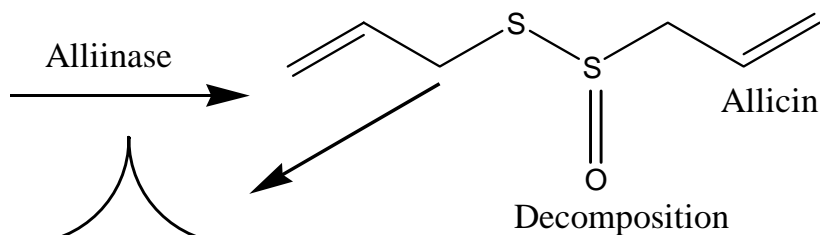
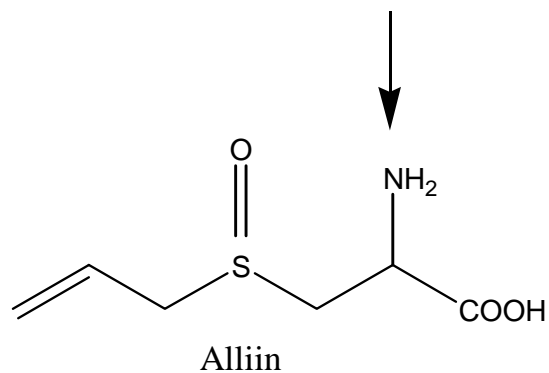
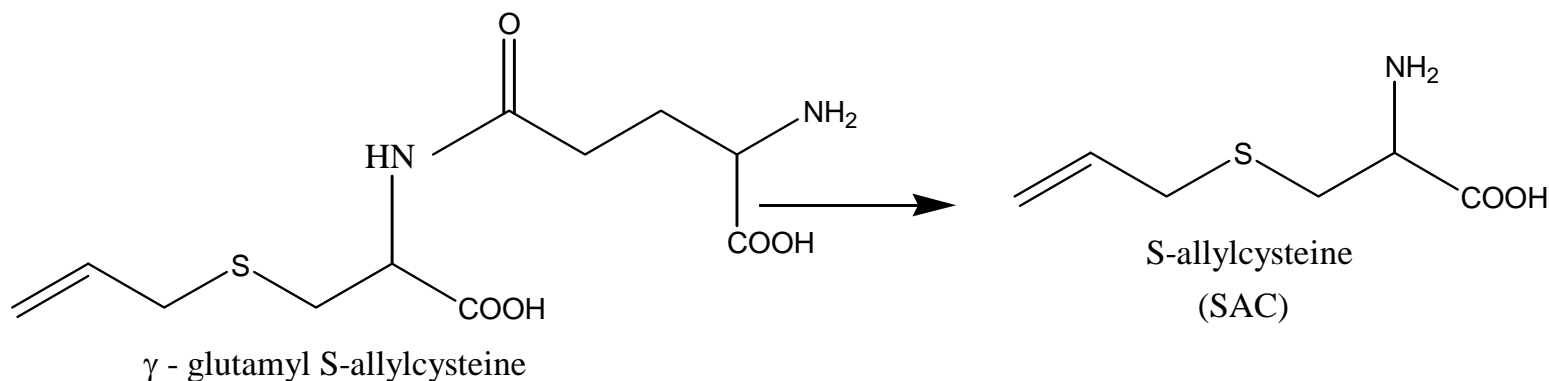


Role of Biomarkers and Surrogate Endpoints: Limitations of Serum Concentrations as a Predictor of Response

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**Department of Pharmacology and University of
Pittsburgh Cancer Institute**

Garlic Derived Sulfur Compounds





Cellular Effects of Garlic Constituents

- **DADS-induced apoptosis (HCT116)**
 - ↑ intracellular calcium (Milner- 1996)
- **DADS-induced G2/M arrest (HCT116)**
 - ↓ Cdk1, ↑ p-Cdk1, ↓ Cdc25C, ↑ Cyclin B (Milner- 1997)
- **DADS-induced apoptosis (MDA-MB-231)**
 - ↑ Bax, ↓ Bcl-xL, and caspase-3 activation (Nakagawa- 2001)



Cellular Effects of Garlic Constituents

- **DADS-induced apoptosis (SH-SY5Y neuroblastoma)-**

↑ ROS, cytochrome c release, JNK activation, and activation of caspase-3 and -9 (Filomeni et al- 2003)

SAMC-induced G2/M arrest and apoptosis induction (SW480, HT-29)-

microtubule depolymerization, JNK and caspase activation (Xiao et al- 2003)



Allium vegetables and risk of prostate cancer: A population-based study.

Hsing AW, Chokkalingam AP, Gao YT, Madigan MP, Deng J, Gridley G, Fraumeni JF Jr.
Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

Epidemiologic and laboratory studies suggest that allium vegetables and garlic constituents have antitumor effects. In a population-based, case-control study conducted in Shanghai, China, we investigated the association between intake of allium vegetables, including garlic, scallions, onions, chives, and leeks, and the risk of prostate cancer. We administered in-person interviews and collected information on 122 food items from 238 case subjects with incident, histologically confirmed prostate cancer and from 471 male population control subjects. **Men in the highest of three intake categories of total allium vegetables (>10.0 g/day) had a statistically significantly lower risk (odds ratio [OR] = 0.51, 95% confidence interval [CI] = 0.34 to 0.76; P(trend)<.001) of prostate cancer than those in the lowest category (<2.2 g/day).** Similar comparisons between categories showed reductions in risk for men in the highest intake categories for garlic (OR = 0.47, 95% CI = 0.31 to 0.71; P(trend)<.001) and scallions (OR = 0.30, 95% CI = 0.18 to 0.51; P(trend)<.001). **The reduced risk of prostate cancer associated with allium vegetables was independent of body size, intake of other foods, and total calorie intake and was more pronounced for men with localized than with advanced prostate cancer.**



Structures of Organosulfides

$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$ Diallyl sulfide (DAS)

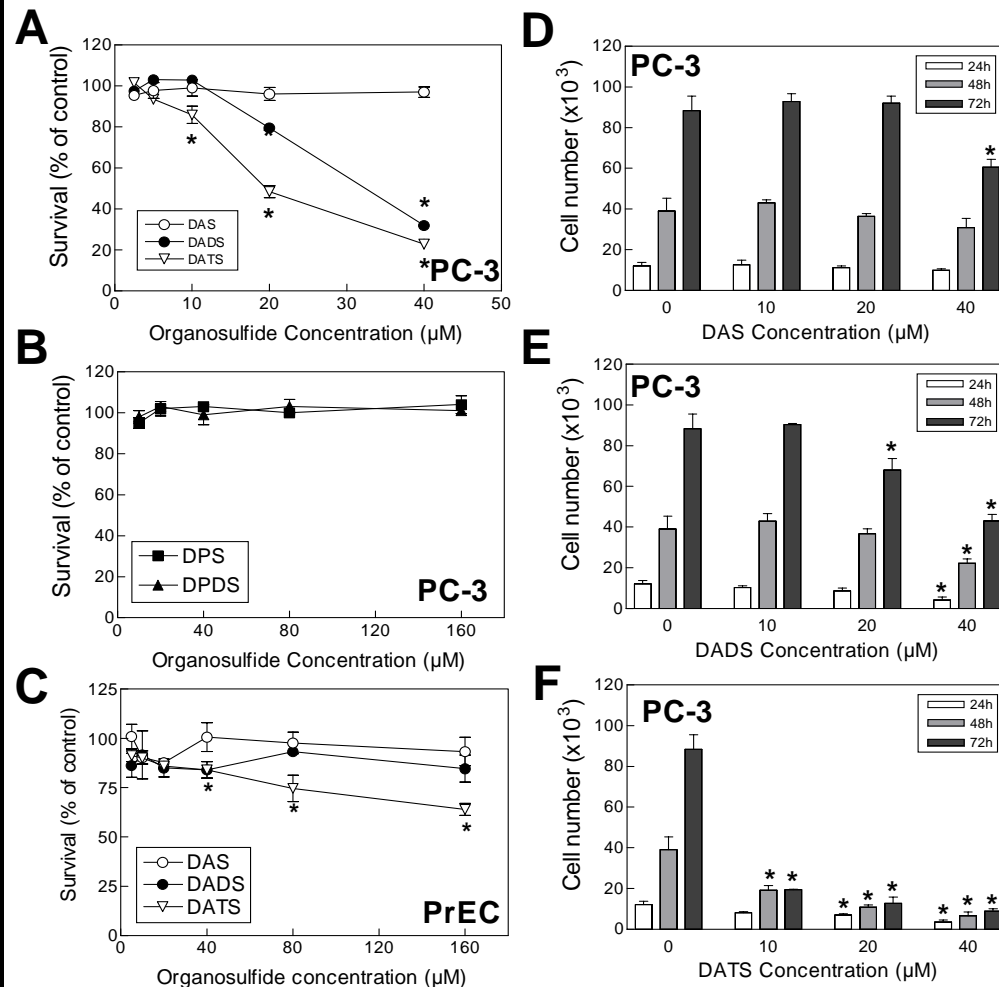
$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$ Diallyl disulfide (DADS)

$\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}-\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$ Diallyl trisulfide (DATS)

$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_3$ Dipropyl sulfide (DPS)

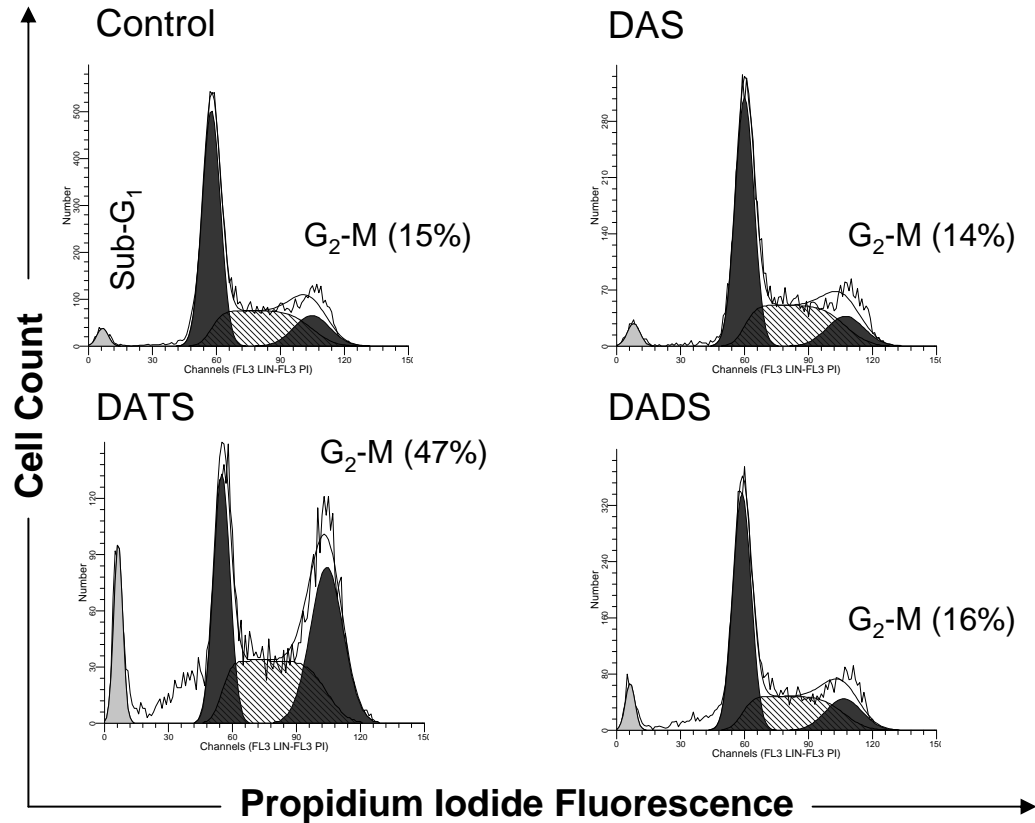
$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_3$ Dipropyl disulfide (DPDS)

DATS Inhibits PC-3 Cell Proliferation

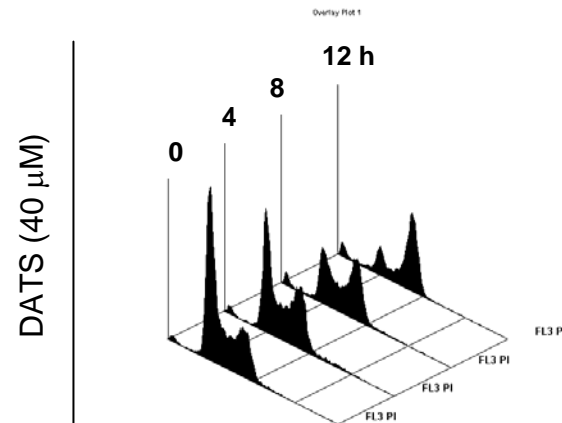
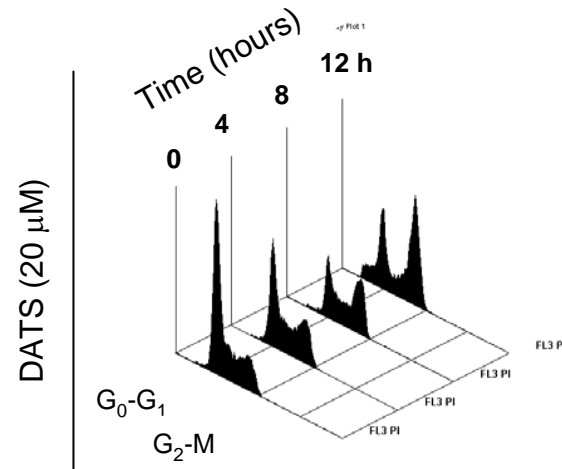


DATS Treated PC-3 Cells Are Arrested in G₂/M Phase of the Cell Cycle

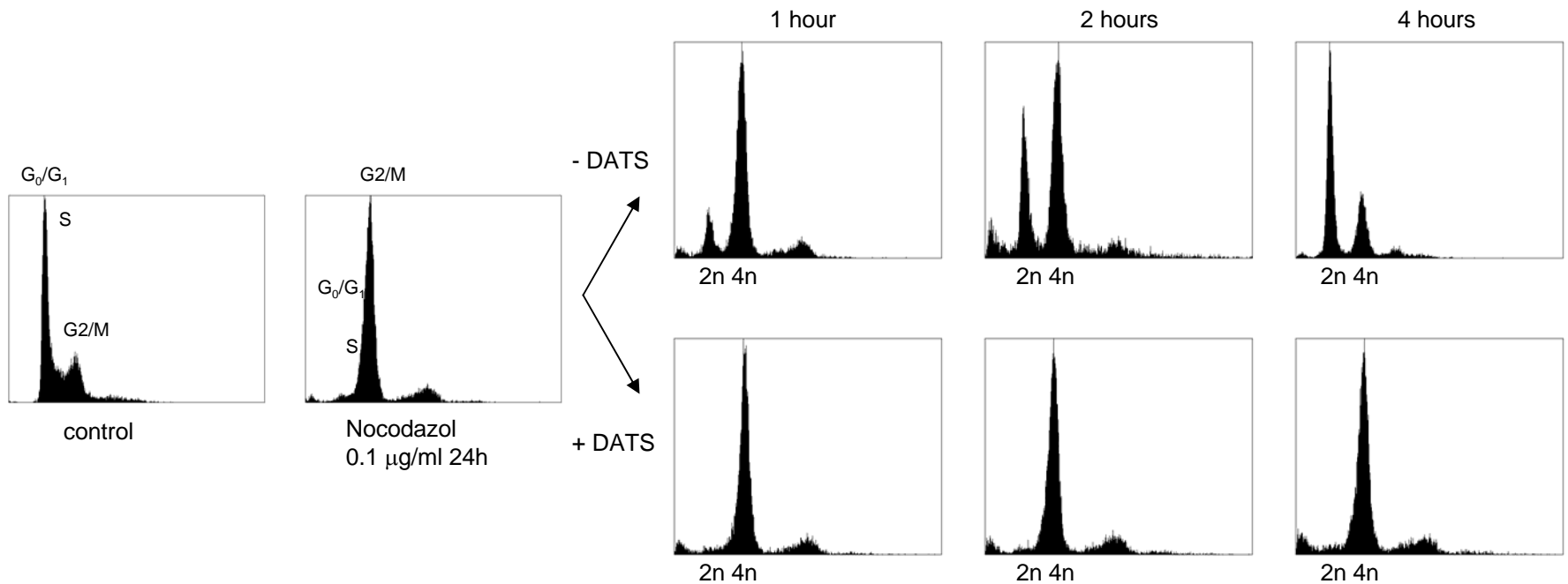
(24 h exposure)



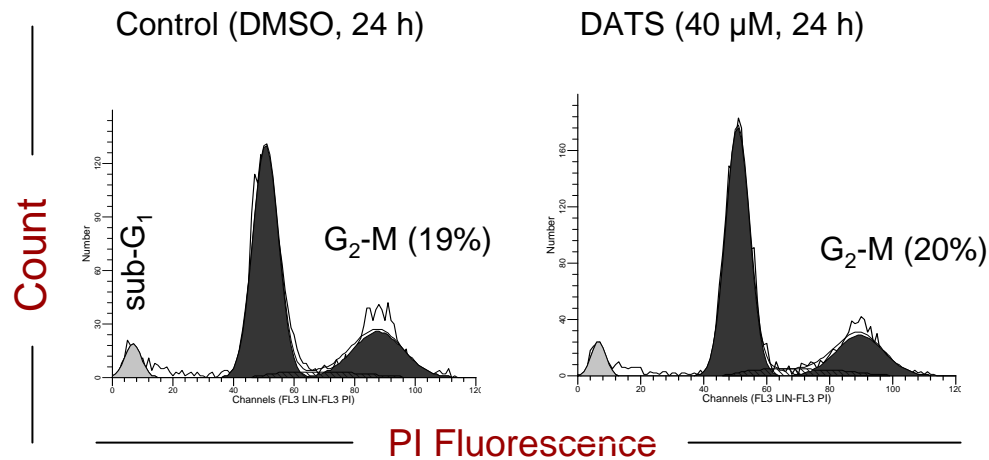
DATS Treated PC-3 Cells Are Arrested in G2/M Phase of the Cell Cycle



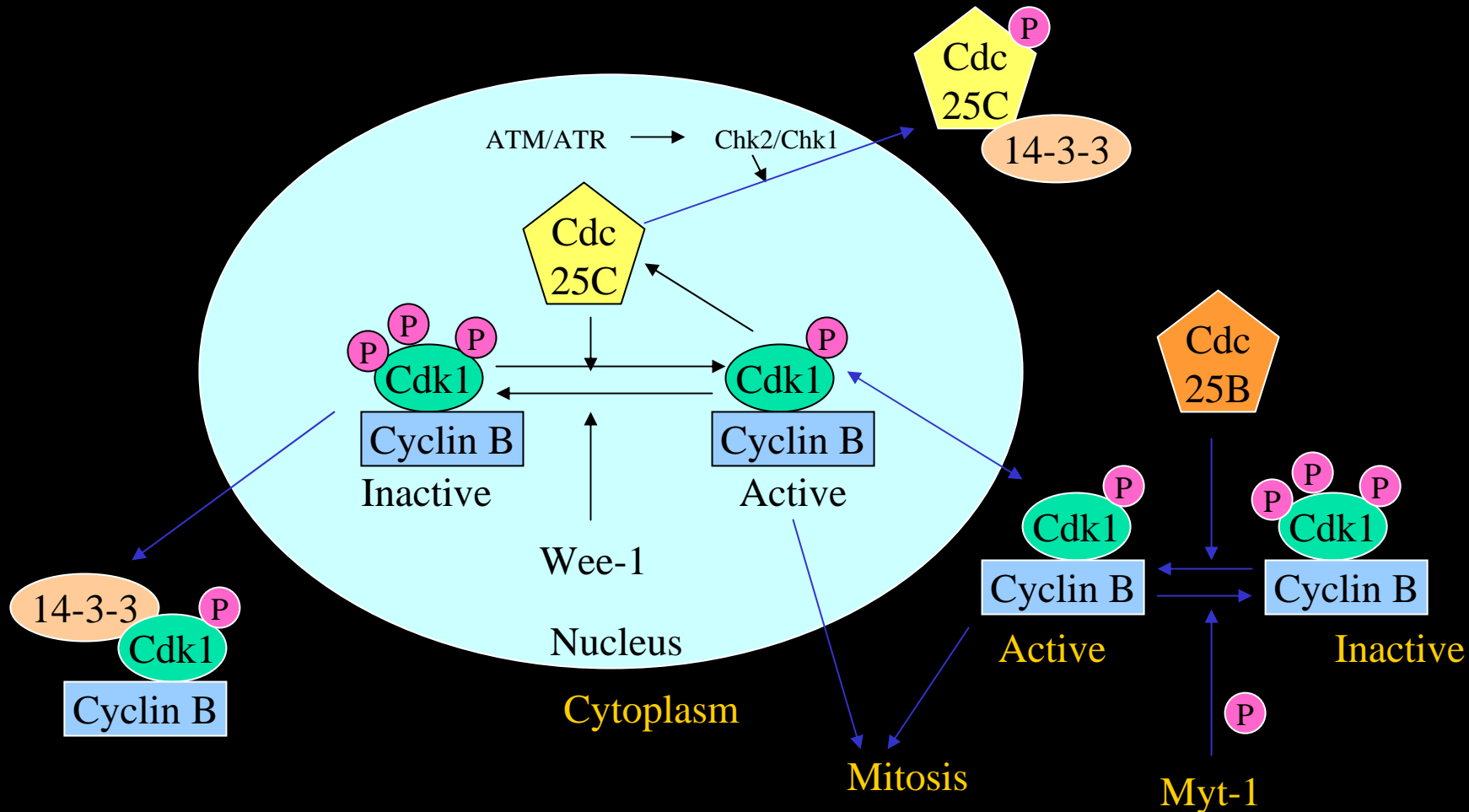
DATS-Induced G2/M Phase Arrest in Synchronized PC-3 Cells

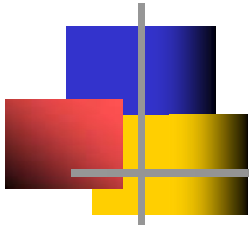


Normal Prostate Epithelial Cells Are Resistant to Cell Cycle Arrest by DATS

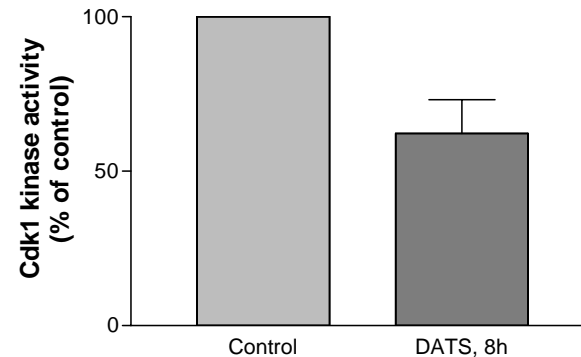
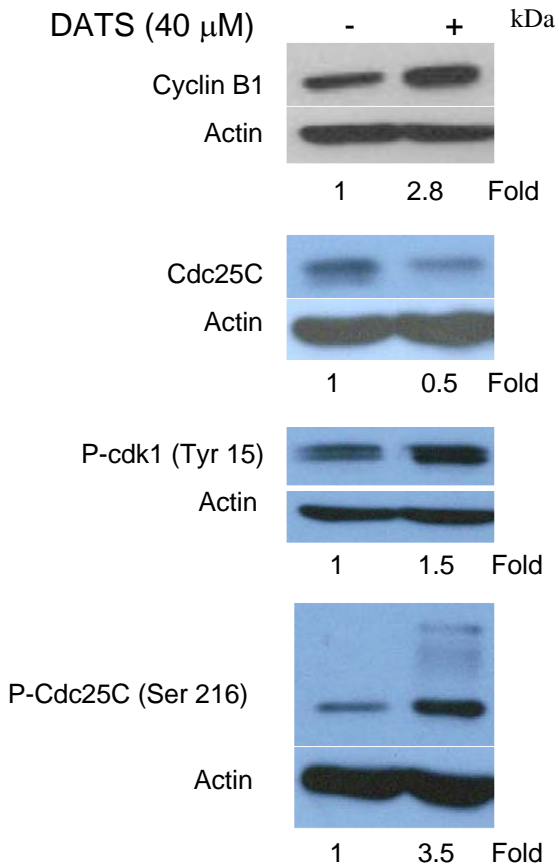


Regulation of G₂/M Transition

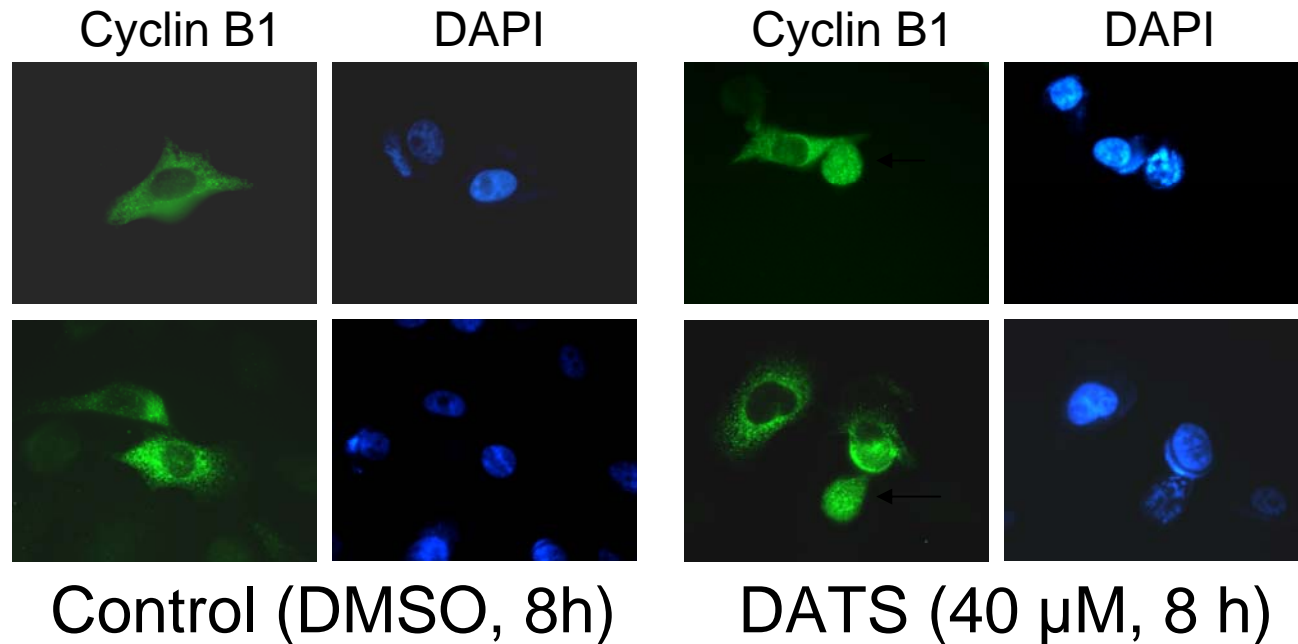




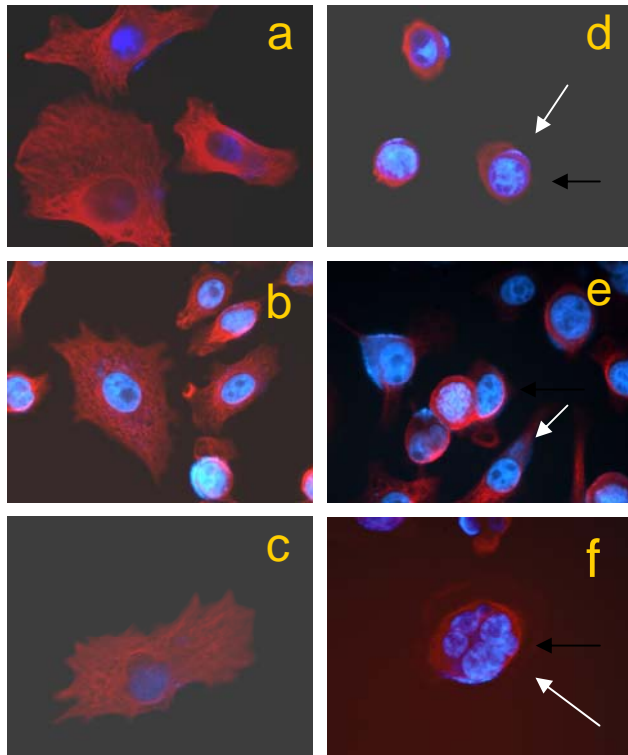
DATS Inhibits Cdk1 activity



Nuclear Accumulation of Cyclin B1 in DATS Treated PC-3 Cells

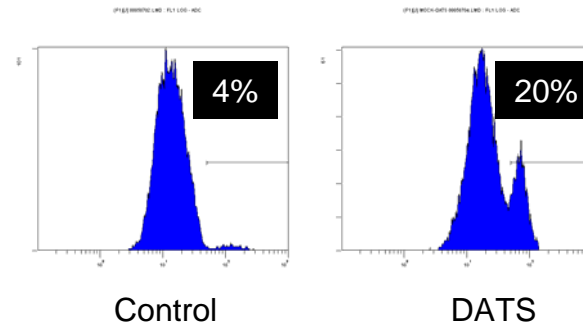
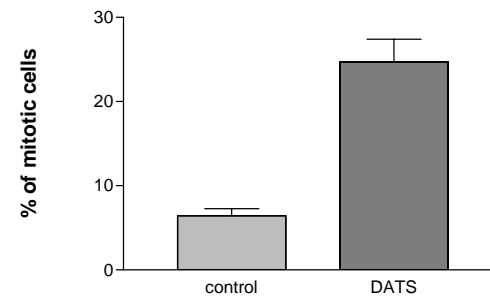


DATS Arrests Cells in Mitosis

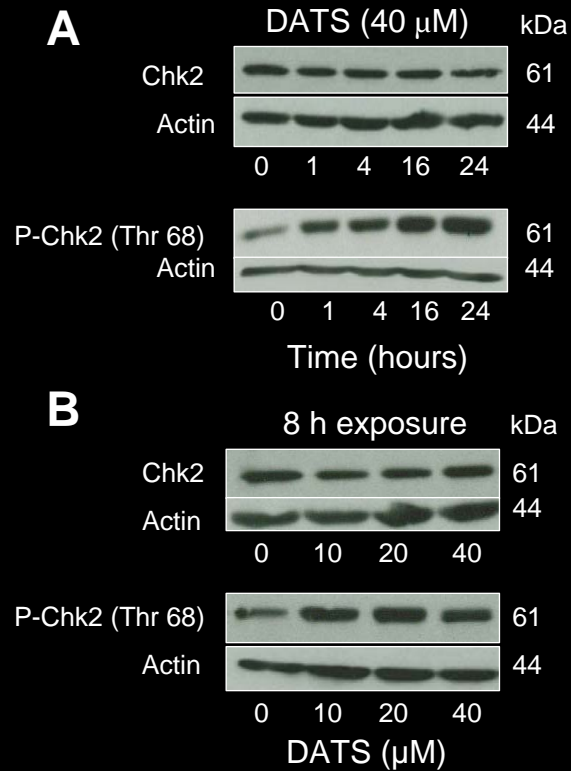


Control

DATS (40 μ M, 8h)



DATS Activates Chk2





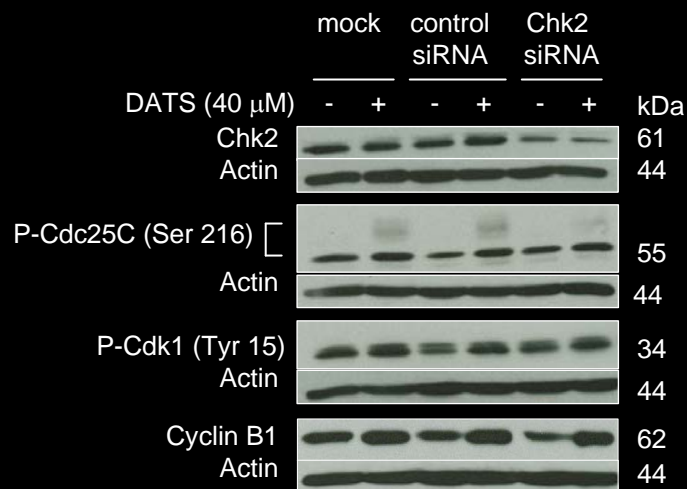
Chk2 Is Dispensable for DATS-Induced G2/M Arrest

Group	Treatment	%G ₀ /G ₁	%S	%G ₂ -M
Mock	DMSO	50 ± 5	20 ± 4	25 ± 2
	DATS	12 ± 1*	18 ± 3	58 ± 5*
Control	DMSO	52 ± 4	17 ± 2	25 ± 1
	DATS	17 ± 3*	17 ± 3	53 ± 7*
Chk2	DMSO	53 ± 4	17 ± 3	25 ± 1
	DATS	18 ± 1*	18	54 ± 1*

Data are mean ± SE (n= 3). Similar results were observed in 2 experiments.

*Significantly different compared with control by one-way ANOVA (p < 0.05).

Chk2 is dispensable for DATS-induced Cdc25C and Cdk1 phosphorylation and Mitotic Arrest



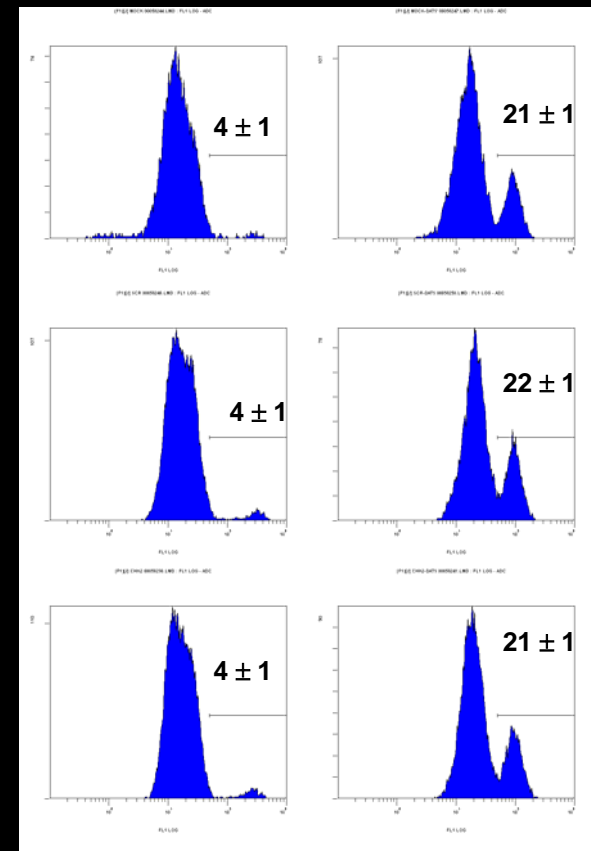
mock

control siRNA

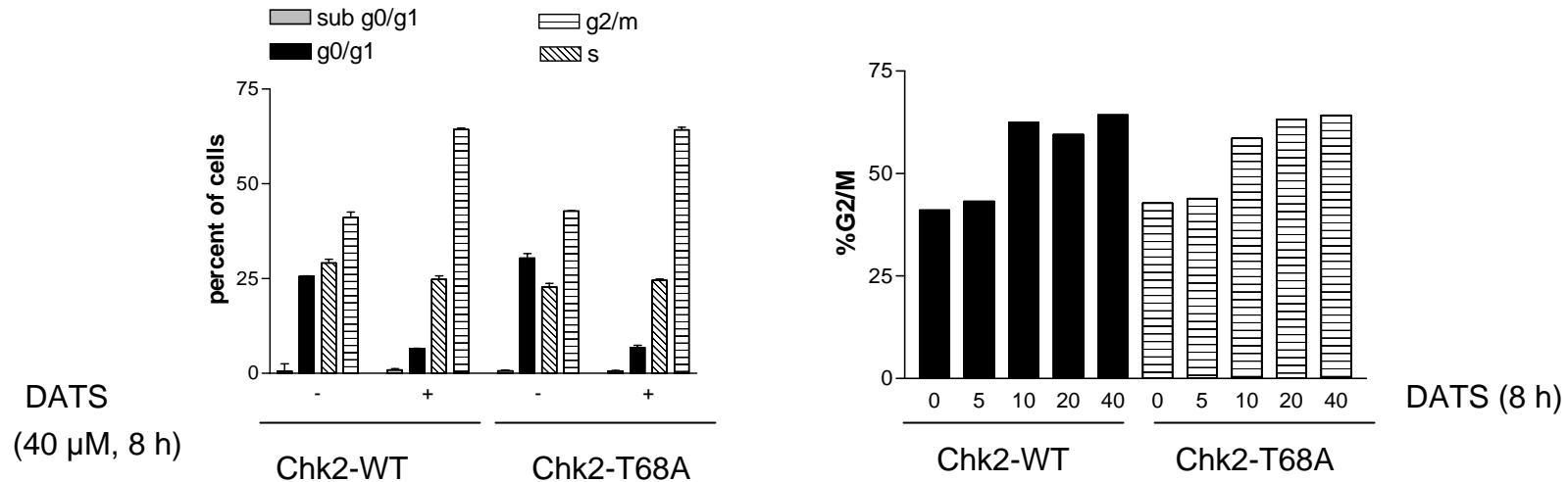
Chk2 siRNA

Control

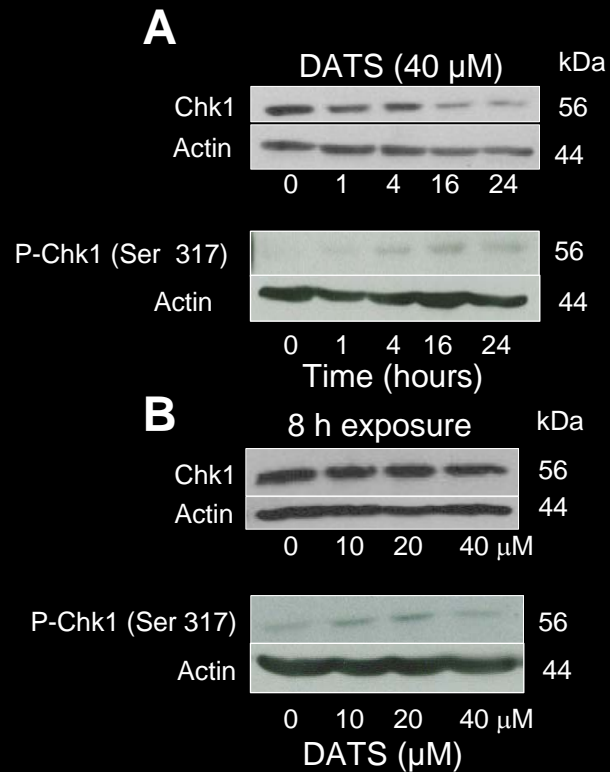
DATS (40 μ M)



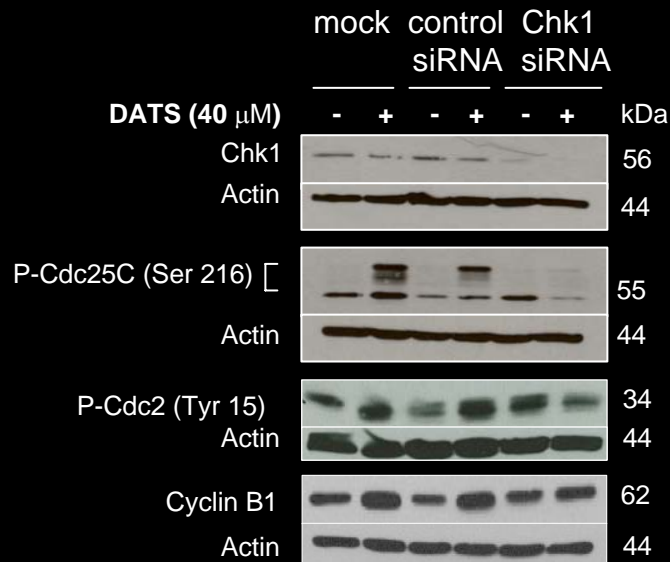
G2/M Arrest in HCT-15 Cells Transfected with WT and Mutant Chk2



DATS Increases Chk1 Phosphorylation



Chk1 Protein Depletion Fails to Overcome DATS-induced G2/M arrest

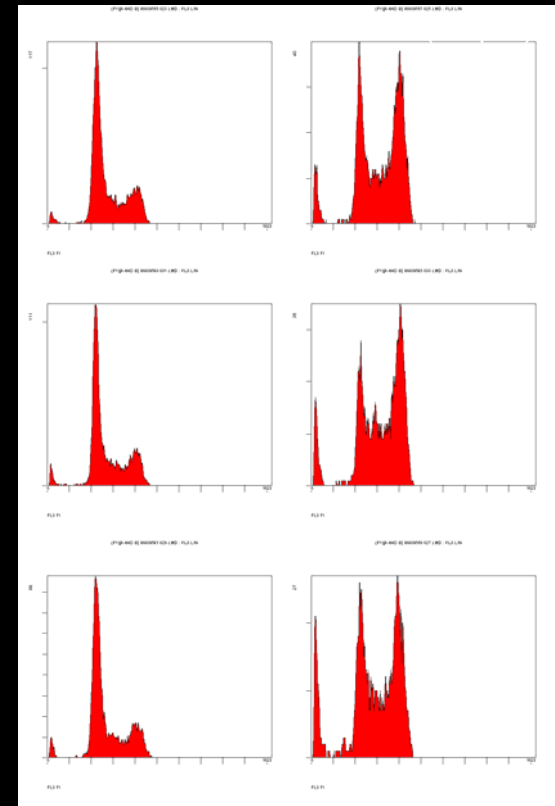


Mock

Control siRNA

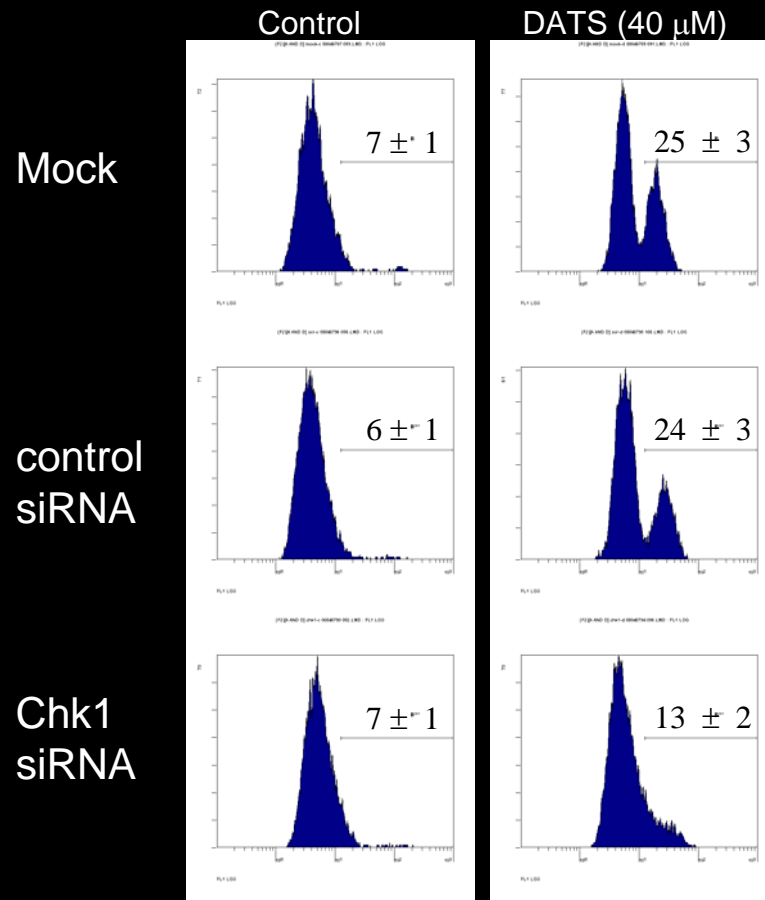
Chk1 siRNA

Cell Count

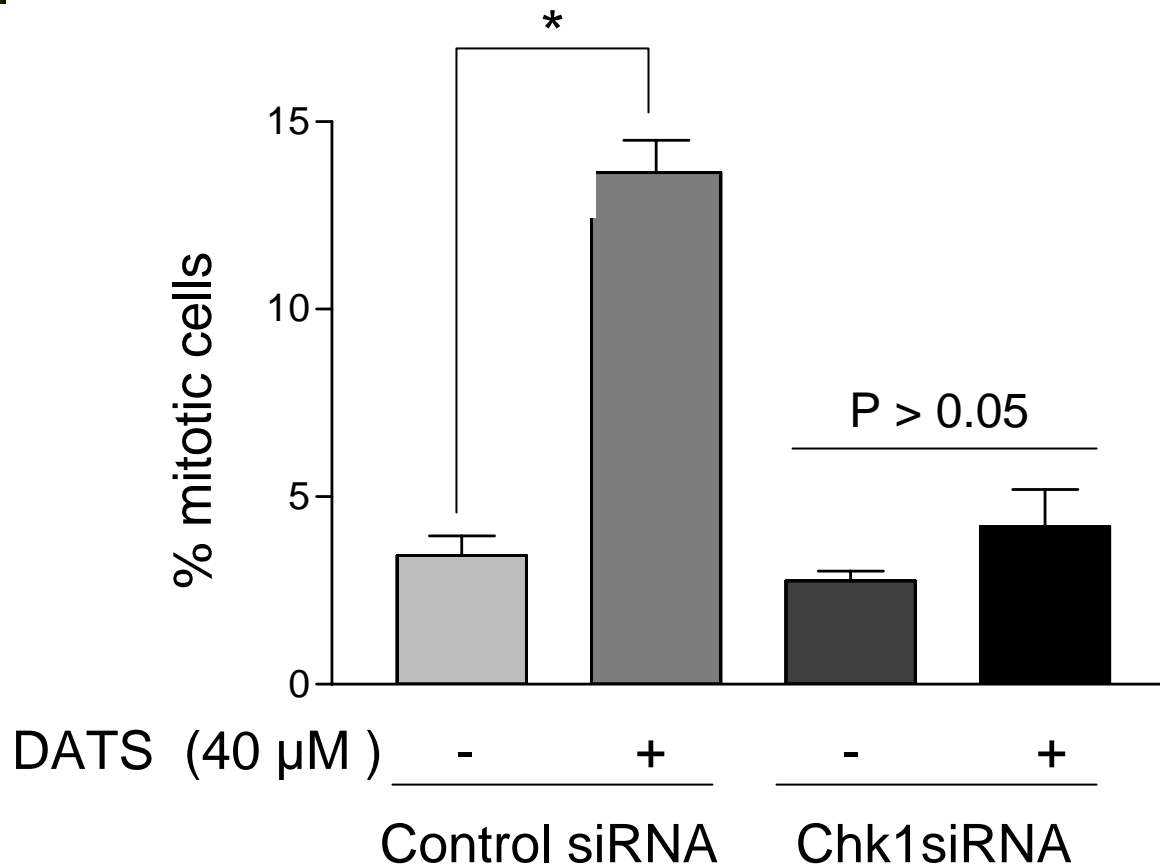


PI Fluorescence

Chk1 Protein Knockdown Attenuates DATS-Induced Histone H3 Phosphorylation



Chk1 Protein Knockdown Inhibits DATS-Induced Mitotic Arrest



*Significantly different compared with control by one-way ANOVA ($P < 0.05$)



Conclusions

- **DATS causes G2 and M phase cell cycle arrest due to:**
 - ROS-dependent degradation of Cdc25C and increased serine-216 phosphorylation of Cdc25C
 - Even though Chk1 protein knockdown inhibits DATS induced phosphorylation of Cdc25C, G₂ arrest is not attenuated.
 - Chk1 depletion inhibits DATS-induced M phase arrest (securin, APC?)
- **DATS induces apoptosis by:**
 - Increasing JNK/ERK mediated phosphorylation of Bcl-2
 - Inhibiting PI3K/Akt pathway



Future Directions

- Mechanism of Chk1-dependent mitotic arrest (securin, Cdc20, APC?).
- Mechanism of DATS induced JNK activation (glutaredoxin/thioredoxin-ASK1?)
- Activity of DATS against prostate tumorigenesis in TRAMP model.
- Toxicological evaluation
- Pharmacokinetics
- Clinical Trial



Thanks to

- CA55589-07, CA76348-06, CA101753-01
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